THE METABOLISM OF BOVINE TUBERCLE BACILLI

STUDIES IN ACID-FAST BACTERIA. XII

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Studies of the nitrogenous metabolism of bovine tubercle bacilli do not appear to have been recorded, although certain peculiar and characteristic progressive changes in the reaction of broth mediums, in which bovine tubercle bacilli have grown, are reported by Theobald Smith[1] and others.

The comprehensive study of Marie Grund[2] on 173 acid-fast viruses, chiefly human and bovine strains of tubercle bacilli, has done much toward explaining the occasional variant which has been reported since Smith's important contribution to this subject.

Bovine tubercle bacilli, generally speaking, differ from human tubercle bacilli in two important particulars. Bovine strains, even in minute doses, are virulent for rabbits, and they produce progressive, titratable alkalinity in bouillon cultures, with or without the addition of glycerol.

Human tubercle bacilli, on the contrary, are practically avirulent for rabbits, and they tend to produce a permanent acid reaction in glycerol broth. There are, however, a few strains of acid-fast bacilli of the tubercle group which appear to be intermediate between the human and bovine types so far as the reaction curve is concerned, and Grund's conclusion that "the glycerin reaction curve is undoubtedly a valuable corroborative evidence of a division of tubercle bacilli into two types, but that its value is lessened by the number of irregular and atypical reactions" may be regarded as expressing the status of the relationship between the reaction curve and the type of tubercle bacillus at the present time.

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[1] Jour. Med. Research, 1904-1905, 13, p. 405.

[2] Studies from the Research Laboratory, Department of Health, New York City, 1911, 6, p. 116.

In a preceding communication[3] on the nitrogenous metabolism of virulent human tubercle bacilli in glycerol broth, it was shown that the reaction curve and the nitrogenous changes in the medium were so related that the conclusion could be drawn that glycerol is an available source of energy for human tubercle bacilli; and as such it spares the nitrogenous constituents of the medium to a considerable degree from bacterial breakdown. The increase in acidity of the medium, together with the practical absence of indications of deamination or proteolysis, are the principal factors on which this assumption was based.

The present investigation is a study of the metabolism of bovine tubercle bacilli. The primary objective is the acquisition of data explanatory of the difference in reaction curves exhibited by human and bovine tubercle bacilli, respectively. That such a distinction exists has long been known. An adequate exposition of the cause has apparently never been made.

The cultures studied were 4—P and M, very virulent for rabbits; G and D, slightly virulent for rabbits. Approximately 1 mg. of P or M inoculated subcutaneously would produce death. Several milligrams of G or D would be required to induce a fatal ending.

The procedure followed was precisely that reported in the study on the metabolism of virulent human tubercle bacilli.[3]

Culture P was inoculated into enough flasks to provide 5 parallel growths for each analysis. Culture M was inoculated in duplicate. Culture D was run in a series of 6 flasks, but only the composite sample, prepared from equal amounts of each flask in the series, is recorded. Culture G was run in a series of 4 flasks. The analyses of the individual flasks, together with the composite, are recorded.

Cultures D and G grew more rapidly and luxuriantly than P and M. This suggests that rapidity of growth is more or less inversely proportional to virulence, an observation which also held true for the human types, studied previously.

The tables show the analytical results.

DISCUSSION

Reaction.—Neutral red: The reaction curves, both of the virulent and avirulent cultures, follow those generally characteristic of bovine types of tubercle bacilli, namely, progressive increases in the titratable alkalinity of the mediums. The maximum degree of the reaction is reached about the sixth week. This is somewhat later than the time of greatest luxuriance in growth, which occurs on or about the fourth week.

[3] Jour. Infect. Dis., 1919, 26, p. 45.

TABLE 1

VIRULENT BOVINE TUBERCLE BACILLUS CULTURE P

Weeks	Flask A					Flask B					Flask C					Flask D					Flask E					Control				
	Neutral Red	Phenolphthalein	Ammonia, mg. per 100 c c	Amino Nitrogen, mg. per 100 c c	Lipase	Neutral Red	Phenolphthalein	Ammonia, mg. per 100 c c	Amino Nitrogen, mg. per 100 c c	Lipase	Neutral Red	Phenolphthalein	Ammonia, mg. per 100 c c	Amino Nitrogen, mg. per 100 c c	Lipase	Neutral Red	Phenolphthalein	Ammonia, mg. per 100 c c	Amino Nitrogen, mg. per 100 c c	Lipase	Neutral Red	Phenolphthalein	Ammonia, mg. per 100 c c	Amino Nitrogen, mg. per 100 c c	Lipase	Neutral Red	Phenolphthalein	Ammonia, mg. per 100 c c	Amino Nitrogen, mg. per 100 c c	Lipase
2	+0.30	000	—2.8	+0.7	—0.05	+0.30	000	—2.8	00	—0.05	+0.30	000	—2.8	00	—0.05	+0.30	000	—2.1	—1.4	—0.05	+0.30	000	—2.8	00	—0.05	—0.10	+0.20	4.9	22.4	0.25
3	000	—0.10	00	+2.1	00	000	—0.10	00	+1.4	00	000	—0.10	00	+2.1	00	000	—0.10	00	+1.4	00	000	—0.10	00	+2.1	00	+0.20	+0.30	4.2	19.6	0.20
4	000	—0.10	+0.7	+0.7	—	000	—0.10	+0.7	+1.4	—	000	—0.10	+0.7	+2.1	—	000	—0.10	00	+1.4	—	000	—0.10	+0.7	+1.4	—	+0.20	+0.30	5.6	19.6	—
5	000	000	00	+0.7	00	—0.10	000	00	00	00	—0.10	000	00	00	00	—0.10	000	00	00	00	—0.10	000	—0.7	+0.7	00	+0.20	+0.30	4.2	21.0	0.15
6	—0.40	000	+0.7	+2.1	00	—0.40	000	+1.4	+2.8	00	—0.40	000	+2.1	+4.9	00	—0.40	000	+1.4	00	00	—0.40	—0.10	+1.4	+2.8	00	+0.10	+0.30	4.9	19.6	0.15
7	—0.50	000	—1.4	+1.4	00	—0.40	—0.10	—1.4	—1.4	00	—0.50	—0.10	—1.4	00	00	—0.50	—0.10	—1.4	00	00	—0.60	—0.10	—2.1	—0.7	00	+0.20	+0.40	4.9	21.0	0.15
8	000	000	+3.5	+1.4	00	000	000	+3.5	+5.6	00	—	—	—	—	—	000	000	+3.5	+5.6	00	—0.10	000	+1.4	+5.6	00	—0.30	+0.30	4.2	22.4	0.20

Phenolphthalein: The increase in titratable alkalinity is less marked, although the change toward the basic side is unmistakable at the height of the development of the cultures. The same relationship between the maximum reaction change and greatest development was also observed in cultures of human tubercle bacilli.[3]

Ammonia.—The ammonia curve of culture P shows an increase from the beginning of the analyses, and this is true, but with greater intensity, for the relatively avirulent cultures G and D. Culture M, on the other hand, is characterized by a minimal increase in ammonia, scarcely greater than can be accounted for by the limits of accuracy of the method. The growth was fairly luxuriant, however, although less rapid than the other strains, indicating that the explanation of the chemical inertness of the organism is to be sought for in some undetermined factor.

TABLE 2

VIRULENT BOVINE TUBERCLE BACILLUS CULTURE M

Weeks	Flask A						Flask B					
	Neutral Red	Phenolphthalein	Ammonia mg. per 100 c c	NH_3 / N %	Amino Nitrogen, mg. per 100 c c	Lipase	Neutral Red	Phenolphthalein	Ammonia mg. per 100 c c	NH_3 / N %	Amino Nitrogen, mg. per 100 c c	Lipase
Control	+0.30	+0.40	4.9		12.6	0.15	+0.30	+0.40	4.9		12.6	0.15
2	—0.10	—0.10	00	00	—1.4	00	—0.10	—0.10	00	00	—1.4	00
3	—0.10	—0.10	+0.7	—0.29	00	00	—0.10	—0.10	00	00	+1.4	00
4	—0.20	—0.10	—1.4	—0.59	.+0.7	00	—0.20	—0.10	—1.4	—0.59	00	00
5	—0.30	—0.20	—1.4	—0.59	—0.7	00	—0.30	—0.20	—0.7	—0.29	00	00
6	—0.50	000	—0.7	—0.29	00	—	—0.50	000	—0.7	—0.29	00	—
7	—0.50	+0.10	—2.1	—0.88	+0.7	00	—0.50	+0.10	—1.4	—0.59	00	00
8	—0.50	—0.10	—1.4	—0.59	00	00	—0.40	—0.10	—1.4	—0.59	00	00
9	—0.60	—0.10	—1.4	—0.59	00	00	—0.20	—0.10	—1.4	—0.59	00	00

Amino Nitrogen.—The amino nitrogen curves, contrary to the human tubercle bacillus amino nitrogen curves, show a slight but unmistakable increase up to the point of maximum development of the culture. After this time, the changes are somewhat irregular, possibly due to unequal rates of autolysis of the bacteria. The general picture is one in which a moderate degree of proteolysis is taking place, contrasting sharply in this respect with human strains, which in glycerol mediums are practically devoid of proteolytic powers.

Total Nitrogen.—A study of the total nitrogen in two of the strains showed a progressive decrease in the amount of this element in the bacteria-free medium. This indicates its incorporation in the bacillary

substance. Recessive changes in the bacteria, leading to partial resolution of nitrogen in the underlying medium, as the autolytic processes became dominant in the cultures, were clearly shown by a gradual increase in soluble nitrogen.

Esterase and Lipase.—The esterase and lipase content of the germ-free broth was practically nil in the virulent strains, but it was distinctly increased in the two avirulent strains. A similar observation was made in the virulent and avirulent human strains.[3]

TABLE 3

AVIRULENT BOVINE TUBERCLE BACILLUS CULTURE D

Weeks	Control				Composite			
	Neutral Red	Ammonia, mg. per 100 c c	Total Nitrogen, mg. per 100 c c	Lipase	Neutral Red	Ammonia, mg. per 100 c c	Total Nitrogen, mg. per 100 c c	Lipase
4	+0.46	13.3	266	0.20	—0.90	+18.2	252	+1.00
5	+0.40	15.7	266	0.20	—1.00	+20.7	259	+1.00
6	+0.40	14.0	273	0.20	—1.20	+21.7	252	+1.10
7	+0.40	14.0	273	0.20	—1.20	+22.4	259	+1.15
8	+0.20	14.0	280	0.20	—1.00	+23.8	252	+1.10
9	+0.20	14.0	287	0.20	—1.10	+23.8	259	+1.15

The composite sample is a mixture of equal portions of the bacillus-free filtrate from six parallel cultures.

The studies on both virulent human and bovine types of tubercle bacilli have shown that these organisms are not very reactive chemically. The lipase and esterase activities also are virtually negative. Less virulent strains grow more rapidly, their chemical activities are greater, and lipase and esterase production becomes a distinct feature of the germ-free filtrates from broth cultures. This difference between virulent and avirulent, or less virulent strains, is of considerable theoretical interest, although nitrogenous metabolic changes fail to reveal the underlying cause. The possibility that tuberculin, a reaction product of growth, may vary somewhat in potency with luxuriance of development suggests itself as a distinct concomitant phenomenon, which may furnish additional information. A communication on this point will be made in the near future.

SUMMARY AND CONCLUSIONS

The neutral red reaction curves of virulent and slightly virulent bovine tubercle bacilli show a small but definite increase in titratable alkalinity, which is progressive. This observation agrees with the typical curves said to be characteristic of the bovine type. The phenol-

TABLE 4

AVIRULENT BOVINE TUBERCLE BACILLUS CULTURE G

Weeks	Control: Neutral Red	Control: Ammonia, mg. per 100 c c	Control: Total Nitrogen, mg. per 100 c c	Control: Lipase	Flask A: Neutral Red	Flask A: Ammonia, mg. per 100 c c	Flask A: $\frac{NH_3}{N}$ %	Flask B: Neutral Red	Flask B: Ammonia, mg. per 100 c c	Flask B: $\frac{NH_3}{N}$ %	Flask C: Neutral Red	Flask C: Ammonia, mg. per 100 c c	Flask C: $\frac{NH_3}{N}$ %
1	+0.20	19.6	350	0.00	—0.90	+2.1	+0.06	—0.80	+6.3	+1.80	—0.80	+8.4	+2.40
2	+0.25	19.6	350	0.00	—0.60	+13.3	+3.80	—0.60	+11.2	+3.20	—0.70	+16.8	+4.80
3	+0.20	19.6	357	0.05	—0.60	+19.6	+5.49	—0.60	+19.6	+5.49	—0.40	+12.6	+3.53
4	+0.25	21.0	364	0.00	—0.90	+16.1	+2.43	—0.90	+7.0	+1.92	—1.00	+9.8	+2.69
5	+0.25	21.0	371	0.00	—1.00	+19.6	+5.28	—1.10	+30.1	+8.11	—1.10	+14.7	+3.97
6	+0.20	20.3	364	0.00	—1.40	+27.3	+7.50	—1.90	+32.9	+9.04	—2.10	+30.1	+8.28

Weeks	Flask D: Neutral Red	Flask D: Ammonia, mg. per 100 c c	Flask D: $\frac{NH_3}{N}$ %	Flask E: Neutral Red	Flask E: Ammonia, mg. per 100 c c	Flask E: $\frac{NH_3}{N}$ %	Composite Sample: Neutral Red	Composite Sample: Ammonia, mg. per 100 c c	Composite Sample: Total Nitrogen, mg. per 100 c c	Composite Sample: $\frac{NH_3}{N}$ %	Composite Sample: Lipase
1	—0.80	+7.7	+2.20	—	—	—	—0.80	+6.3	350	1.80	+0.05
2	—0.70	+19.6	+5.60	—0.70	+16.8	+4.8	—0.70	+15.4	308	5.00	+1.10
3	—0.60	+25.9	+7.25	—0.60	+24.5	+6.86	—0.60	+21.7	315	6.90	+1.15
4	—1.00	+15.4	+4.23	—0.90	+14.0	+3.85	—0.90	+14.0	301	1.80	+1.15
5	—1.10	+31.5	+8.50	—1.10	+22.4	+6.04	—1.10	+24.5	315	7.78	+1.55
6	—1.70	+29.4	+8.08	—1.70	+30.8	+8.46	—1.80	+31.5	294	10.70	+1.50

The composite sample is an analysis of a mixture of equal parts of the bacillus-free filtrates from flasks A, B, C, D and E, inclusive.

phthalein reaction curve is less pronounced in this respect, but the period of greatest luxuriance in growth is accompanied by a slight increase in alkalinity. The ammonia curves are suggestive of increased deamination as the culture develops, but unlike the human tubercle bacillus curve, the trend is constantly toward an increase. Presumably the latter part of the ammonia rise is associated rather with autolysis of the bacteria than with an increase in protein utilization when the culture is decadent. There is also an increase in soluble nitrogen during the autolytic period, which in a measure supports this view.

Culture M is characterized by a minimal change in the ammonia curve, but evidence drawn from the amino nitrogen curve leads to the conclusion that this variation from the other strains is merely one of degree, not of type.

Generally speaking, the ammonia curves and curves of deamination of the bovine tubercle are the reverse of this characteristic of virulent human tubercle bacilli. The former increase with luxuriance of development in the culture, the latter decrease under the same conditions.

Increased ammonia production suggests increased action on the proteolytic constituents of the medium, and decreased ammonia indicates the virtual absence of proteolysis.

The amino-acid curves are in harmony with this view. In bovine strains the amount of soluble amino-acid increases with the development of the bacteria, and the maximum of amino-acid and of growth practically coincide in point of time. The exact reverse is true for those strains of virulent human tubercle bacilli which have been studied.

The three noteworthy features in the metabolism of the bovine tubercle bacilli herein studied — progressively alkaline reaction in glycerol mediums, increase in ammonia (or deamination), and parallel increase of amino-acids — point definitely to two significant facts: First, that the character of the metabolism of these organisms, both virulent and avirulent, is distinctly proteolytic in glycerol broth, and, secondly, that glycerol does not appear to spare the protein constituents of the broth from bacterial breakdown. Glycerol, in other words, is not a source of energy for bovine tubercle bacilli. On the contrary, glycerol appears to be an important source of energy for human tubercle bacilli, and the marked differences between the metabolism curves of the human and bovine types, including the Smith reaction curves, appear to depend on this very definite and simple fact.

The limited number of observations herein recorded do not, of course, permit of too great generalization on this point, but the chemical evidence, so far as it goes, taken into consideration with the reaction curves characteristc of the human and bovine types, are absolutely in harmony and appear to justify the conclusion that glycerol is a source of energy to human tubercle bacilli, but it is not a source of energy to bovine tubercle bacilli.

The development of an acid reaction in glycerol cultures of human tubercle bacilli and the absence of chemical evidence of proteolysis indicate that the human type can ferment glycerol. The development of an alkaline reaction in glycerol cultures of bovine tubercle bacilli, together with the unmistakable increase of deamination and cleavage of protein to amino-acids, indicates equally strongly that bovine tubercle bacilli cannot or do not ferment glycerol. This appears to be the true explanation for the observed differences in reaction curves of the two types of organisms.